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Innate Immunity: The Worm Fights Back

Dispatch

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Innate immunity is an evolutionarily ancient defense system that enables animals and plants to resist invading microorganisms. Recent studies have demonstrated the existence of innate immune responses in *Caenorhabditis elegans*.

The nematode *Caenorhabditis elegans* is susceptible to infection by a variety of fungal and bacterial pathogens [1]. In other animals and plants, such infections are known to be challenged by innate immune defenses. These detect conserved pathogen-specific cellular components such as lipopolysaccharide (LPS) and peptidoglycan. They then counter the invasion with various responses which limit the infection or kill the invading microorganism. In *Drosophila*, for example, the Toll receptor is activated on infection by fungi and Gram-positive bacteria, initiating an intracellular signaling cascade that culminates in the activation of Rel/NFκB transcription factors. These factors then promote expression of antimicrobial peptides [2]. Signaling from Toll-like receptors also constitutes a major arm of the mammalian innate immune response [3], suggesting common evolutionary roots for these defense mechanisms.

Surprisingly, however, Toll-dependent defense appears absent from *C. elegans* [4]. Not only are genes encoding a number of components of the cascade missing from the worm genome — including, critically, a Rel/NFκB homolog — but also deletion of the components that are present was found not to alter nematode resistance to a number of pathogens [4]. Apparently then, *C. elegans* does not use this otherwise conserved pathway for immune defense. Indeed, until recently, there was no clear evidence for a defense system in the worm, despite the fact that it normally inhabits an environment teeming with potential pathogens. The data presented in two recent papers [5,6], however, demonstrate both the existence of a conserved innate immune signaling pathway in *C. elegans* and the induction of various genes in response to infection.

Reasoning that an animal lacking a component of its innate immune system would be hypersensitive to infection, Kim and coworkers [5] sought mutant worms displaying enhanced susceptibility to pathogens, a phenotype they termed Esp. They did this in the context of a *Pseudomonas aeruginosa* infection of the *C. elegans* intestine, which normally kills worms after approximately 34 hours. By rescuing eggs from the uteri of dead infected mothers who had succumbed prematurely to this infection, the group identified a number of Esp mutants. Two of these, *esp-2* and *esp-8*, show 100% and 90% mortality, respectively, after 31 hours of exposure to the pathogen, while no dead wild-type animals are observed at this time point. The use of a green fluorescent protein-labeled form of the bacterium confirmed that in both these mutants there is precocious accumulation of the pathogen in the intestine compared with wild-type [5].

The mutations that cause hypersensitivity in these mutant strains were found in two known genes, one encoding a mitogen activated protein (MAP) kinase kinase kinase, *nsy-1* (*esp-8*), and the other, its downstream MAP kinase kinase, *sek-1* (*esp-2*). As the mammalian MAP kinase kinases to which SEK-1 is most homologous, MKK3/MKK6, specifically activate the p38 family of MAP kinases, the *C. elegans* p38 homologs were suggested as the downstream kinases in this cascade. The inhibition of one of these, PMK-1, by RNA interference (RNAi) was shown to result in hypersensitivity to infection.

Kim *et al.* [5] provided further evidence for a MAP kinase cascade involving NSY-1, SEK-1 and PMK-1 (Figure 1) by demonstrating that the two Esp mutants have markedly lower levels of p38 MAP kinase activity than that of wild-type worms in the presence of the pathogen [5]. The established role of a MAP kinase

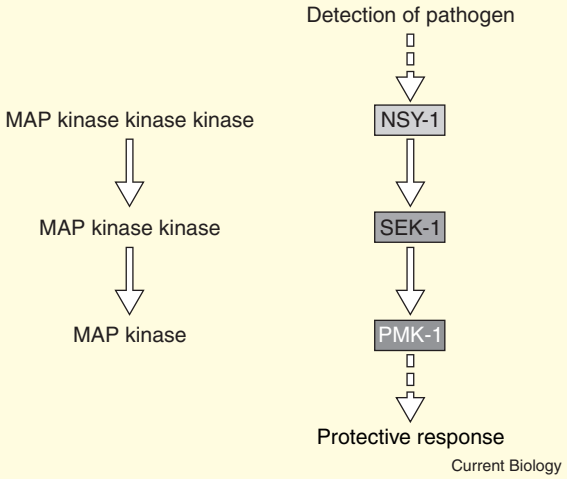

Figure 1. MAP kinase cascade in *C. elegans* immunity.

MAP kinase cascades are ancient signal transduction pathways used by diverse organisms in many physiological processes. MAP kinase kinase kinases phosphorylate MAP kinase kinases which, in turn, activate MAP kinases by phosphorylation. In *C. elegans*, NSY-1, SEK-1 and PMK-1 define a MAP kinase cascade that is essential for defense against bacterial infection. Loss of function of any of these kinases increases worm susceptibility to infection by both *Pseudomonas aeruginosa* and *Enterococcus faecalis* [5]. At present it is not clear how the worm detects these pathogens to cause activation of the p38 MAP kinase cascade and the nature of the protective response invoked by this activation is equally unknown.

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pathway such as this in the mammalian cellular immune response to LPS [7], and the implication of such signaling in the modulation of insect immune response [8], suggest that *C. elegans* does indeed possess an innate immune signaling pathway that is both conserved and ancient.

The Esp mutants were identified for their hypersensitivity to infection by *P. aeuruginosa*, a Gram-negative pathogen. They were also tested for susceptibility to infection by the Gram-positive bacterium *Enterococcus faecalis*, and found to be hypersensitive to this pathogen as well [5]. This finding suggests that the p38 MAP kinase pathway is involved in broad-spectrum antibacterial defense and increases interest in the question as to what the downstream targets of this cascade might be.

The other recent work in this area [6] asks whether, like other animals, *C. elegans* responds to infection with the induction of defense genes. Such genes include those encoding antimicrobial peptides. Although a number of peptides resembling defensins and amoebapore peptides — antibacterial peptides of *Entamoeba histolytica* — have been described in the worm [9,10], the inducibility of their expression has not been demonstrated.

Using microarrays, Mallo *et al.* [6] assessed the transcriptional response of approximately 7500 *C. elegans* genes to infection by another intestinal pathogen, *Serratia marcescens*. RNA from worms that had been exposed to this Gram-negative bacterium for 24 and 48 hours was compared with that from animals grown on the standard laboratory diet of *Escherichia coli* strain OP50. Seven genes showed greater than two-fold induction at both time points. Of these, three appear to be nematode-specific and only one has a clear homolog, being similar to vertebrate gastric lipases. Lipases are also induced after infection of *Drosophila* and may act directly against invading microorganisms [11]. The remaining three genes encode proteins containing lectin domains, motifs which in both invertebrates and vertebrates are known to be involved in recognition of infecting microorganisms [12,13]. These results confirm that *C. elegans* does respond to infection with the induction of specific genes, but it remains to be seen whether their protein products are directly involved in defense in this context.

Mallo *et al*. [6] also specifically examined genes encoding lysozymes, which are known to act both alone and synergistically with antibacterial peptides in innate immune responses. They found that *lys-1*, *lys-7* and *lys-8* all showed induction following infection [6]. These *C. elegans* lysozymes are homologous to those of *E. histolytica*, suggesting that they may act in synergy with the previously mentioned amoebapore peptides to eliminate invading microorganisms [14].

Remarkably, a comparison of the results of this array experiment with one previously performed to identify genes under the control of the transforming growth factor-β-related signalling molecule DBL-1, revealed that two of the genes induced on infection by *S. marcescens* are also regulated by this growth factor [6,15]. Prompted by this observation, a *dbl-1* loss-of -function mutant was tested for response to infection and found to be hypersensitive [6]. Indeed this mutant can even succumb to the usually harmless feeding strain, *E. coli* OP50. Interestingly, the *Drosophila* homolog of DBL-1, Decapentaplegic (Dpp), has recently been found to be induced in response to infection [16]. So, despite the absence of a Toll pathway, there is increasing evidence for overlap between other branches of pathogen response in flies and worms.

Together these two studies [5,6] provide the first evidence of a conserved innate immune defense system in *C. elegans* and demonstrate the inducibility of defense genes in response to infection. With the ever-growing number of known *C. elegans* pathogens, and the tractability of this organism for genetic studies, the worm is likely to provide new insights into mechanisms of innate immune defense.

References

1. Ewbank, J.J. (2002). Tackling both sides of the host-pathogen equation with *Caenorhabditis elegans*. Microbes Infect. *4*, 247–256.
2. Tzou, P., De Gregorio, E. and Lemaitre, B. (2002). How *Drosophila* combats microbial infection: a model to study innate immunity and host-pathogen interactions. Curr. Opin. Microbiol. *5*, 102–110.
3. Medzhitov, R. (2001). Toll-like receptors and innate immunity. Nat. Rev. Immunol. *1*, 135–145.
4. Pujol, N., Link, E.M., Liu, L.X., Kurz, L.C., Alloing, G., Tan, M.W., Ray, K.P., Solari, R., Johnson, C.D. and Ewbank, J.J. (2001). A reverse genetic analysis of components of the Toll signalling pathway in *Caenorhabditis elegans*. Curr. Biol. *11*, 809–821.
5. Kim, D.H., Feinbaum, R., Alloing, G., Emerson, F.E., Garsin, D.A., Inoue, H., Tanaka-Hino, M., Hisamoto, N., Matsumoto, K., Tan, M.W. *et al.* (2002). A conserved p38 MAP kinase pathway in *Caenorhabditis elegans* innate immunity. Science *297*, 623–626.
6. Mallo, G.V., Kurz, C.L., Couillault, C., Pujol, N., Granjeaud, S., Kohara, Y. and Ewbank, J.J. (2002). Inducible antibacterial defense system in *C. elegans*. Curr. Biol. *12*, 1209–1214.
7. Dong, C., Davis, R.J. and Flavell, R.A. (2002). MAP kinases in the immune response. Annu. Rev. Immunol. *20*, 55–72.
8. Han, Z.S., Enslen, H., Hu, X., Meng, X., Wu, I.H., Barrett, T., Davis, R. and Ip, Y.T. (1998). A conserved p38 mitogen-activated protein kinase pathway regulates *Drosophila* immunity gene expression. Mol. Cell. Biol. *18*, 3527–3539.
9. Kato, Y., Aizawa, T., Hoshino, H., Kawano, K., Nitta, K. and Zhang, H. (2002). *abf-1* and *abf-2*, ASABF-type antimicrobial peptide genes in *Caenorhabditis elegans*. Biochem. J. *361*, 221–230.
10. Bányai, L. and Patthy, L. (1998). Amoebapore homologs of *Caenorhabditis elegans*. Biochim. Biophys. Acta *1429*, 259–264.
11. De Gregorio, E., Spellman, P.T., Rubin, G.M. and Lemaitre, B. (2001). Genome-wide analysis of the *Drosophila* immune response by using oligonucleotide microarrays. Proc. Natl. Acad. Sci. U.S.A. *98*, 12590–12595.
12. Wilson, R., Chen, C. and Ratcliffe, N.A. (1999). Innate immunity in insects: the role of multiple, endogenous serum lectins in the recognition of foreign invaders in the cockroach, *Blaberus discoidalis*. J. Immunol. *162*, 1590–1596.
13. Fujita, T. (2002). Evolution of the lectin-complement pathway and its role in innate immunity. Nat. Rev. Immunol. *2*, 346–353.
14. Leippe, M. (1999). Antimicrobial and cytolytic polypeptides of amoeboid protozoa – effector molecules of primitive phagocytes. Dev. Comp. Immunol. *23*, 267–279.
15. Mochii, M., Yoshida, S., Morita, K., Kphara, Y. and Ueno, N. (1999). Identification of transforming growth factor-β-regulated genes in *Caenorhabditis elegans* by differential hybridisation of arrayed cDNAs. Proc. Natl. Acad. Sci. U.S.A. *96*, 15020–15025.
16. Irving, P., Troxler, L., Heuer, T.S., Belvin, M., Kopczynski, C., Reichhart, J.M., Hoffmann, J.A. and Hetru, C. (2001). A genome-wide analysis of immune responses in *Drosophila*. Proc. Natl. Acad. Sci. U.S.A. *98*, 15119–15124.